AD NUMBER

AD806443

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; AUG 1958. Other requests shall be referred to US Army Medical Research Lab., Fort Knox, KY.

AUTHORITY

US Army Medical Research Lab. ltr dtd 26 Feb 1970

REPORT NO. 357

THE INFLUENCE OF THE CHEMICAL COMPOSITION OF THE MEDIUM ON THE RESTORATION OF VIABILITY OF ULTRAVIOLET IRRADIATED ESCHERICHIA COLI, STRAIN B*

by

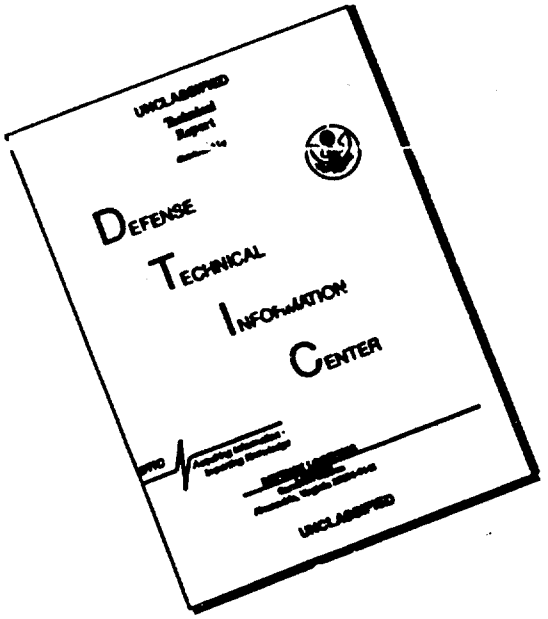
E. E. Woodside, C. R. Goucher
and W. Kocholaty

from

Biochemistry Department
US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY

*Subtask under Biological and Medical Aspects of Ionizing Radiation, USAMRL Project No. 6-59-08-014, Subtask, Biochemistry of Blood Corpuscles under Irradiation and Other Stresses.

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

Report No. 357
Project No. 6-59-08-014
Subtask USAMRL S-2
MEDEA

ABSTRACT

THE INFLUENCE OF THE CHEMICAL COMPOSITION OF THE MEDIUM ON THE RESTORATION OF VIABILITY OF ULTRAVIOLET IRRADIATED ESCHERICHIA COLI, STRAIN B

OBJECT

The purpose of this study was to elucidate certain environmental influences which alter the viability of the ultraviolet irradiated bacterium, E. coli B.

RESULTS AND CONCLUSIONS

Suspensions of E. coli B when irradiated with ultraviolet light and subsequently incubated in the dark show a spontaneous rise in viable cell count. This increase in viability is demonstrable over a wide range of ultraviolet inactivation levels and is most pronounced with high doses of inactivating radiation. The increase in viability is dependent on the composition of the medium in which the cells are suspended. Potassium and phosphate ions as well as glucose were found to be essential for maximal dark reactivation.

RECOMMENDATIONS

None.

Submitted 1 July 1958 by:

E. E. Woodside, Biochemist
C. R. Goucher, Biochemist
W. Kocholaty, Biochemist

APPROVED: H. Jensen
H. JENSEN
Head, Biochemistry Department

APPROVED: Floyd A. Odell
FLOYD A. ODELL
Technical Director of Research

APPROVED: Joseph R. Blair
JOSEPH R. BLAIR
Lt Colonel, MC
Commanding

THE INFLUENCE OF THE CHEMICAL COMPOSITION OF THE MEDIUM ON THE RESTORATION OF VIABILITY OF ULTRAVIOLET IRRADIATED ESCHERICHIA COLI STRAIN B

I. INTRODUCTION

A simple relationship does not appear to exist between the radiation dose absorbed by a suspension of bacteria (1, 2, 3, 4, 5, 6, 7) and the fraction or organisms killed. The quantity (8) as well as the quality (6, 9) of radiation and post irradiation treatment control the inactivation levels associated with the absorption of a given amount of radiant energy by bacteria. The demonstration of spontaneous dark recovery of cells of E. coli B following ultraviolet irradiation (10) has been confirmed subsequently (1, 2, 8).

The present study examines the influence of certain environmental conditions on the viability of E. coli B following ultraviolet irradiation. These investigations reveal that glucose, phosphate, and potassium ions profoundly influence the degree of post irradiation survival of this organism.

II. EXPERIMENTAL

A. Materials and Methods

1. Organisms. E. coli, strains B and B/r were kindly supplied by Dr. Max Delbrück, Kerckhoff Lab., Inst. Tech., Pasadena, California, and by Dr. Alexander Hollaender, Oak Ridge Nat. Lab., Oak Ridge, Tennessee.

2. Media. Bacto nutrient broth (pH 6.8) and Monod's (11) synthetic medium (adjusted to pH 7.4 with KOH) with 0.2% glucose were used as growth media. Plating media were the same as those used for growth with the addition of 1% Bacto agar (12). An aliquot of a sterile 20% glucose solution was added to either the sterile synthetic growth or plating medium to give a final concentration of 0.2%. The buffer solution used was M/15 monobasic potassium phosphate adjusted to pH 7.40 with KOH.

3. Preparation of cultures. 0.5 ml of an 8-hr old culture was inoculated into 100 ml of a synthetic growth medium. After 20 hrs growth on a reciprocal shaker at 37° C, the cells were centrifuged and washed two times with cold M/15 phosphate buffer. The cells were

suspended in buffer to an optical density of 0.79 at 575 mμ (a Coleman Jr. spectrophotometer was used). This suspension was aerated for 20 hrs on a reciprocal shaker at 37° C, centrifuged once and brought to 0.79 optical density with cold buffer to give a standard stock solution. This stock solution was further diluted with phosphate buffer to a concentration of approximately 2×10^6 organisms/ml in the ultraviolet radiation experiments.

4. Irradiation. All ultraviolet irradiations were carried out at room temperature in either M/15 phosphate buffer, or 0.1M trishydroxyaminomethane (THAM) buffer of pH 7.4. The source of the ultraviolet radiation was a 15 watt GE germicidal lamp, placed 56 cm from the sample. A constant voltage regulator was inserted into the power line and the lamp was allowed to burn for 30 min before irradiation of the sample. The depth of all samples was adjusted to 4.5 mm (13).

5. Post-irradiation treatment and plating technique. In all dark incubations nine parts of the irradiated samples were diluted with one part of the desired substrate (0.2% glucose). Necessary precautions were observed in illuminating the room during the dark incubation periods (14). The pour plate method was used throughout, observing the special precautions recommended by Snyder (15). The poured plates were maintained at room temperature for 90 min and then placed in a 37° C incubator. Incubation time for Monod's agar plates was 48 hrs, for nutrient broth agar plates 24 hrs.

6. Calculations. The calculations proposed by Kelner were used (8, 16, 17). In addition, calculations of the logarithmic growth phase were carried out according to Beckhorn (18).

III. RESULTS AND DISCUSSION

The survival of bacteria, incubated in various media at 37° C for definite time intervals, is expressed as a fraction of the number of viable cells in either potassium, sodium phosphate, or THAM buffer. The necessity for expressing the results in relation to the corresponding buffer control is evident upon examination of the survival curves of unirradiated control cells suspended in these buffers (Fig. 1).

A. Effect of Monod's and of Nutrient Broth Medium on the Dark Survival of U.V. Irradiated E. coli B

The composition of the plating medium has a decisive effect on the survival of X- or gamma-irradiated cells of *E. coli* (19, 20); therefore,

the dark recovery obtained with E. coli when plated on a synthetic glucose medium was compared with recoveries obtained on nutrient broth agar. From the data presented in Table 1, it is evident that following ultraviolet irradiation, nutrient broth agar effects a protection at zero time compared with cells plated on Monod's glucose agar. These higher initial recoveries at time zero could be attributed either to meeting the nutritional requirements needed by some radiation induced mutants or to the protective effect of lower pH of the plating media[1] as reported by Weatherwax (3). In contrast to the beneficial effects of nutrient broth, the increase in viable cell count observed after 1 to 3 hrs incubation and plating on Monod's medium with glucose is of a much greater magnitude when compared with incubation in nutrient broth for identical time intervals. This difference cannot be attributed to either protection by added essential nutritives or by pH differences of the plating and/or culture medium.

B. The Increase in Viable Cell Count in Suspensions of E. coli B During Incubation in the Dark After Irradiation with Ultraviolet Light

For an evaluation of the protective effects obtained in media of varying composition, any change in the cell count observed in the irradiated buffer controls must be taken into consideration. Consequently, the results are expressed as relative recoveries based on a ratio of the cell count observed in a protective mixture to that of the buffer controls. When cells of E. coli B suspended in Monod's medium with glucose are irradiated with varying doses of ultraviolet light and subsequently incubated in the same medium at 37° C in the dark an unusual increase in the viable cell count occurs, the pattern of which is illustrated in Figure 2. This increase persists within a wide range of initial survival levels (0.1 - 63%).[2] At 28 and 63% initial survival, the lag phase is extended to 3 hrs as compared to the half hr lag phase of an unirradiated culture. At doses of irradiation resulting in 0.1 and 6% survival, the lag phase of the culture is extended to 5 or 6 hrs. Since the cells are suspended in a medium which is capable of supporting normal growth, one may expect a proportionality between the initial cell count and growth. However, as the initial cell count decreases the extent of the unusual rise in viable cells upon incubation in the dark actually increases. This inverse relationship is suggestive of a progressive reactivation of the irradiated bacteria resulting from prolonged dark incubation in the specified growth medium.

[1] The nutrient broth and agar had a pH of 6.8 while the pH of the synthetic glucose broth and agar was 7.4.

[2] "Initial survival" is the fraction of viable organisms found immediately following irradiation.

C. Influence of Various Cations on the Dark Survival of Cells at Various Intervals Following the Exposure to Ultraviolet Radiation

The effect of the various ions and of ion combinations, present in Monod's medium with glucose as the only carbon source, on the pattern of survival of E. coli B after irradiation and dark incubation was studied at 0.1% survival level. Figure 3 (b-e) shows that the omission of Fe^{++}, Mg^{++}, Ca^{++}, and NH_4^+ from Monod's medium has no effect on the increase of viable cells observed during dark incubation when compared with the complete growth medium (Fig. 3-a). Furthermore, the slopes of the dark reactivation curves show a rapid logarithmic increase of viable cells during the first to the third hr of incubation which is greater than the slope of the line representing the increasing viable count of unirradiated suspensions.

D. Establishment of Basal Requirements Necessary for the Increased Viable Cell Count of Irradiated Cell Suspensions

Paralleling the maximum restoration, observed in the Fe^{++} free growth medium with 0.2% glucose, is the dark recovery obtained with cells incubated only in M/15 potassium phosphate buffer with 0.2% glucose - Figure 3-g. The necessity of glucose in stimulating dark survival is evidenced by the inability of U. V. irradiated cells to maintain viability in a medium deficient in glucose (c.f. Fig. 3-f). Since irradiated cells suspended in sodium phosphate-glucose (Fig. 3-g and f) show only 1/3 of the dark recovery compared to potassium phosphate-glucose suspensions, it is evident that not only glucose and phosphate, but also potassium ions are essential for the maximum dark recovery.

Further additional evidence for the essentiality and possibly interdependence of potassium, phosphate, and glucose for the dark recovery phenomenon is furnished by the results obtained in substituting THAM buffer (pH 7.4) for Monod's salt medium during the irradiation and dark incubation period (Fig. 4). Although THAM buffer alone has a deleterious effect on E. coli B upon prolonged incubation (Fig. 1), dark incubation of ultraviolet irradiated cells in THAM buffer containing potassium and phosphate ions as well as glucose results in protection (Fig. 4-a). Similar results, as shown in Figure 4-b, were obtained with the following salt and/or glucose combinations: phosphate, or glucose, or potassium, or potassium plus phosphate, or potassium plus glucose. Data obtained in this study tend to show that the spontaneous dark reactivations occur as a result of alternations in rates of processes in which each of the ions investigated may contribute to cell death and cell recovery

IV. SUMMARY

Suspensions of E. coli B when irradiated with ultraviolet light and subsequently incubated in the dark, show a spontaneous rise in cell count. This increase in viability is demonstrable over a wide range of exposures to ultraviolet light and is greater with high doses of radiation. The increase in viability is dependent also on the composition of the medium in which the cells are suspended. Potassium and phosphate ions and glucose were found to be essential for maximal dark reactivation.

V. RECOMMENDATIONS

None.

VI. REFERENCES

1. Ellison, S. A., B. F. Erlanger, and P. Allen. The chemical reversal of ultraviolet effects on bacteria. J. Bact. 69: 536, 1955.

2. Charles, R. L., and L. M. Zimmerman. Dark reactivation in ultraviolet irradiated Escherichia coli. J. Bact. 71: 611, 1956.

3. Weatherwax, R. S. Reactivation of ultraviolet irradiated Escherichia coli. J. Bact. 72: 329, 1956.

4. Ogg, J. E., H. I. Adler, and M. R. Zelle. Protection of Escherichia coli against ultraviolet irradiation by catalase and related enzymes. J. Bact. 72: 494, 1956.

5. Kelner, A. Photoreactivation of ultraviolet irradiated Escherichia coli, with special reference to the dose reduction principle and to ultraviolet-induced mutation. J. Bact. 58: 511, 1949.

6. Loofbourow, J. R. Effects of ultraviolet radiation on cells. Growth Symposium 12: 75, 1948.

7. Latarjet, R., L. R. Caldas, B. Miletic and P. Morenne. Chemical restoration in microorganisms. Brit. J. Radiol. 27: 54, 1954.

8. Heinmets, F., J. J. Lehman, W. W. Taylor, and R. H. Kathan. The study of factors which influence metabolic reactivation of the ultraviolet inactivated Escherichia coli. J. Bact. 67: 511, 1954.

9. Barner, H. D. and S. S. Cohen. The relation of growth to the lethal damage induced by ultraviolet irradiation in Escherichia coli. J. Bact. 71: 149, 1956.

10. Woodside, E. E. and W. Kocholaty. A comparison of ultraviolet sensitivity and photoactivation phenomena in radiation resistant and radiation sensitive strains of Escherichia coli. AMRL Report No. 141, Ft. Knox, Ky., 1954.

11. Monod, J. T. and M. Jolit. Sur la reactivation de bacteries steriliseer par la rayonnenment ultraviolet. Compt. Rend. Acad. Sci. 229: 557, 1949.

12. Roberts, R. B. and E. Aldous. Recovery from ultraviolet irradiation in Escherichia coli. J. Bact. 57: 363, 1949.

13. Heinmets, F. and W. W. Taylor, Jr. Photobiological studies on Escherichia coli at low temperature. J. Bact. 62: 447, 1951.

14. Dulbecco, R. Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. J. Bact. 59: 329, 1950.

15. Snyder, T. L. The relative errors of bacteriological plate counting methods. J. Bact. 54: 641, 1947.

16. Kelner, A. Effect of visible light on the recovery of Streptomyces Griseus conidia from ultraviolet irradiation injury. Proc. Nat. Acad. Sci. 35: 74, 1949.

17. Kelner, A. Growth, respiration, and nucleic acid synthesis in ultraviolet-irradiated and in photoreactivated Escherichia coli. J. Bact. 65: 252, 1953.

18. Beckhorn, E. J. Photoreversal of the lengthening effect of ultraviolet radiations on the bacterial lag period. J. Bact. 63: 687, 1952.

19. Stapleton, G. E., A. J. Sbarra, and A. Hollaender. Some nutritional aspects of bacterial recovery from ionizing radiations. J. Bact. 70: 7, 1955.

20. Anderson, E. H. and D. Billen. The effect of temperature on X-ray induced mutability in Escherichia coli. J. Bact. 70: 35, 1955.

TABLE 1

EFFECTS OF NUTRIENT BROTH AND MONOD'S MEDIUM ON THE DARK SURVIVAL OF U.V. IRRADIATED *E. COLI* B

ALL CELLS INCUBATED IN THE DARK WITH MONOD'S MEDIUM PLUS 0.2% GLUCOSE					
Hours Dark Incubation Before Plating	0	1	3	4	5
Plated on Monod's	1.88×10^3	8.50×10^3	1.19×10^4	1.08×10^4	3.40×10^3
Plated on Nutrient Broth Agar	5.60×10^3	1.56×10^4	3.70×10^3	5.10×10^3	4.20×10^3
Ratio: $\frac{\text{Monod's Count}}{\text{Nut. Broth Count}}$	0.336	0.531	3.22	2.12	0.810
ALL CELLS PLATED ON MONOD'S MEDIUM PLUS 0.2% GLUCOSE					
Dark Incubation with Monod's	3.14×10^3	4.02×10^4	7.15×10^4	6.55×10^4	4.81×10^4
Dark Incubation with Nutrient Broth	7.28×10^3	5.30×10^3	5.10×10^3	1.35×10^4	7.47×10^4
Ratio: $\frac{\text{Monod's Count}}{\text{Nut. Broth Count}}$	0.432	7.58	14.0	4.85	0.644

Numbers refer to viable organisms per ml.

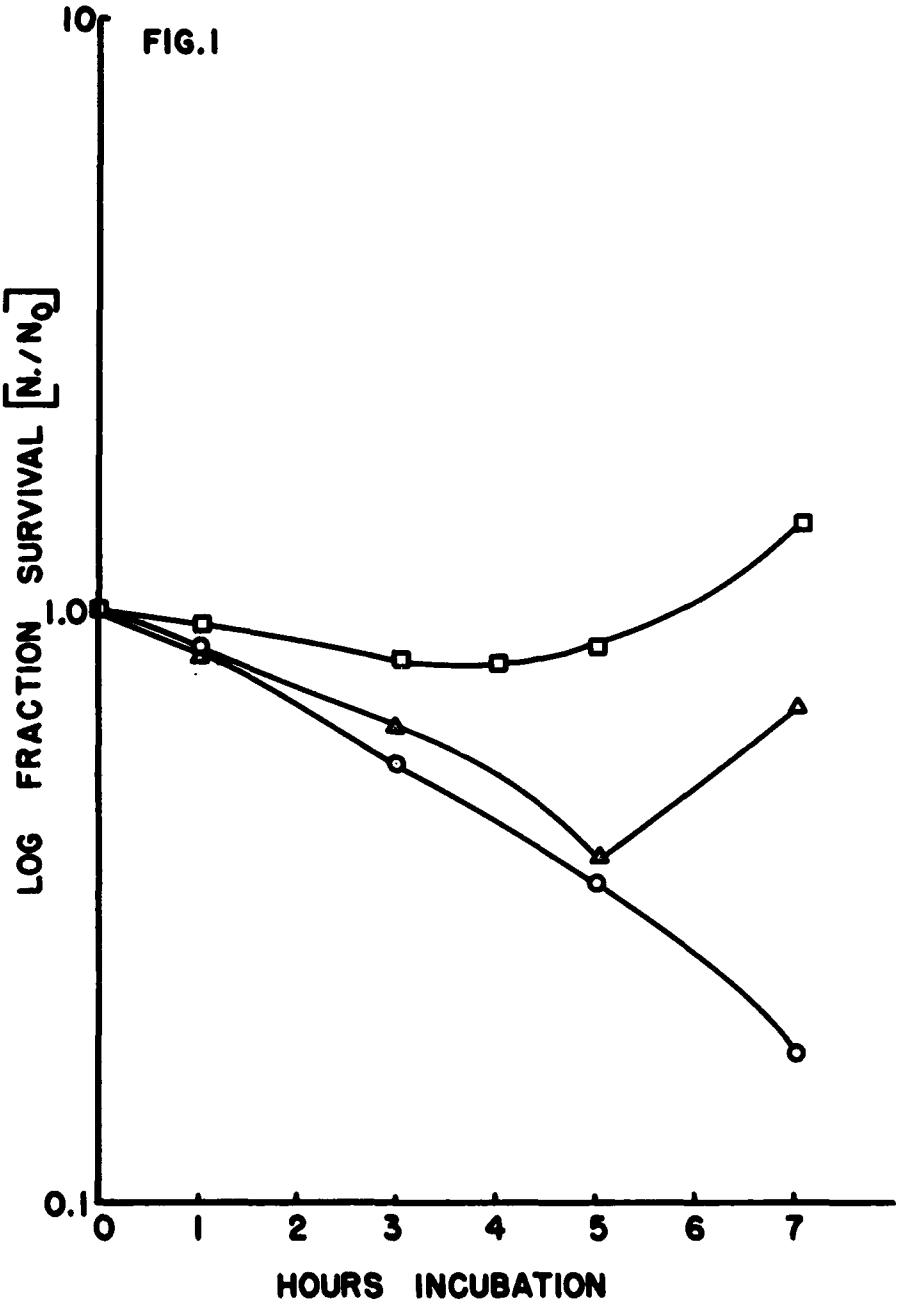


Fig. 1. Effect of prolonged 37° C dark incubation on non-irradiated E. coli B in various buffer systems.

○ - THAM Buffer; △- Sodium Phosphate Buffer;
□ - Potassium Phosphate Buffer

FIG. 2

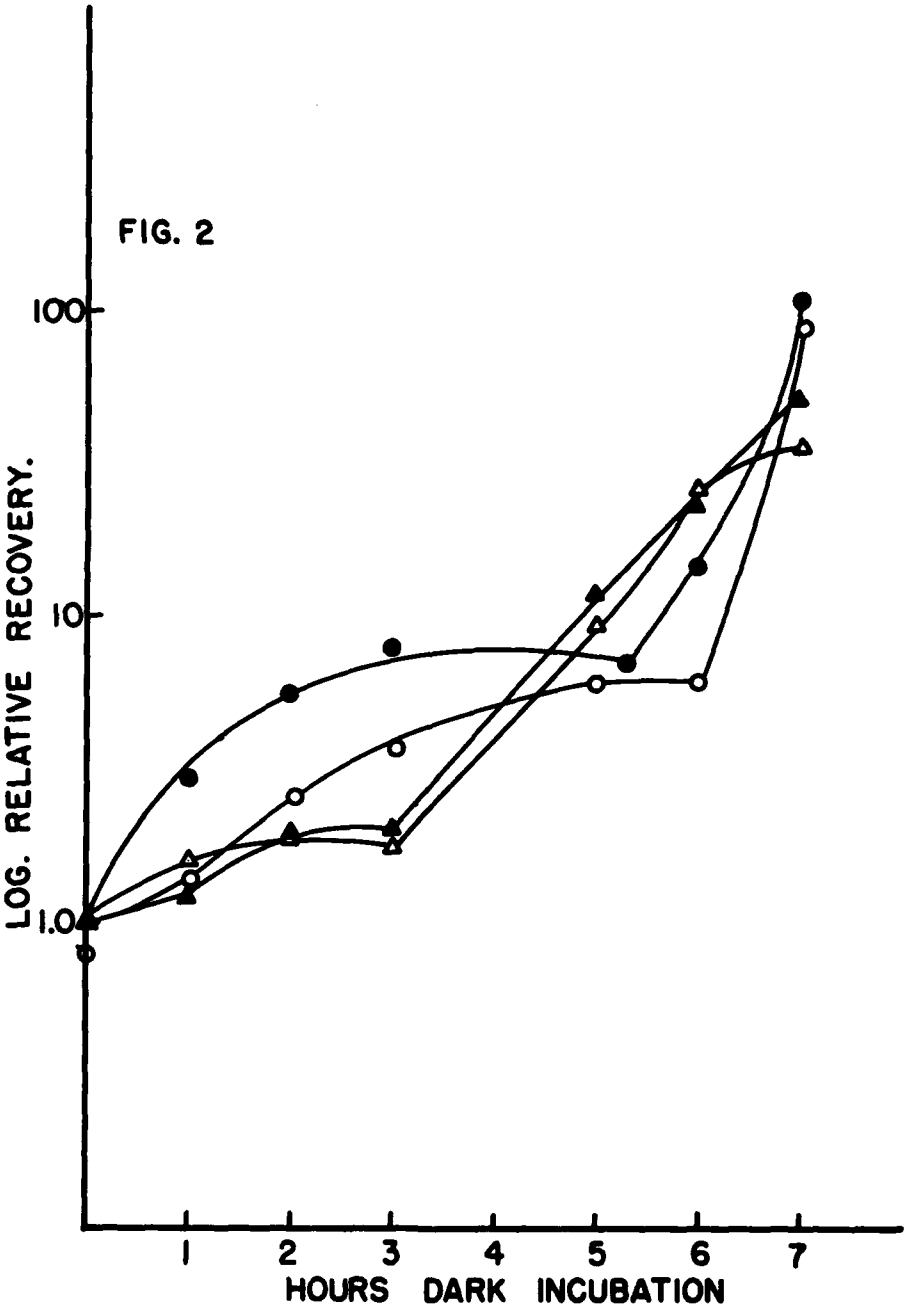


Fig. 2. Effect of varying ultraviolet doses on the spontaneous increase of E. coli B.

● - 0.12 ± 0.06% Initial Survival; ○ - 5.78 ± 0.74%; △ - 28.1 ± 4.1%; ▲ - 63.4 ± 5.0%. Incubation Medium: Monod's with 0.2% Glucose at 37° C.

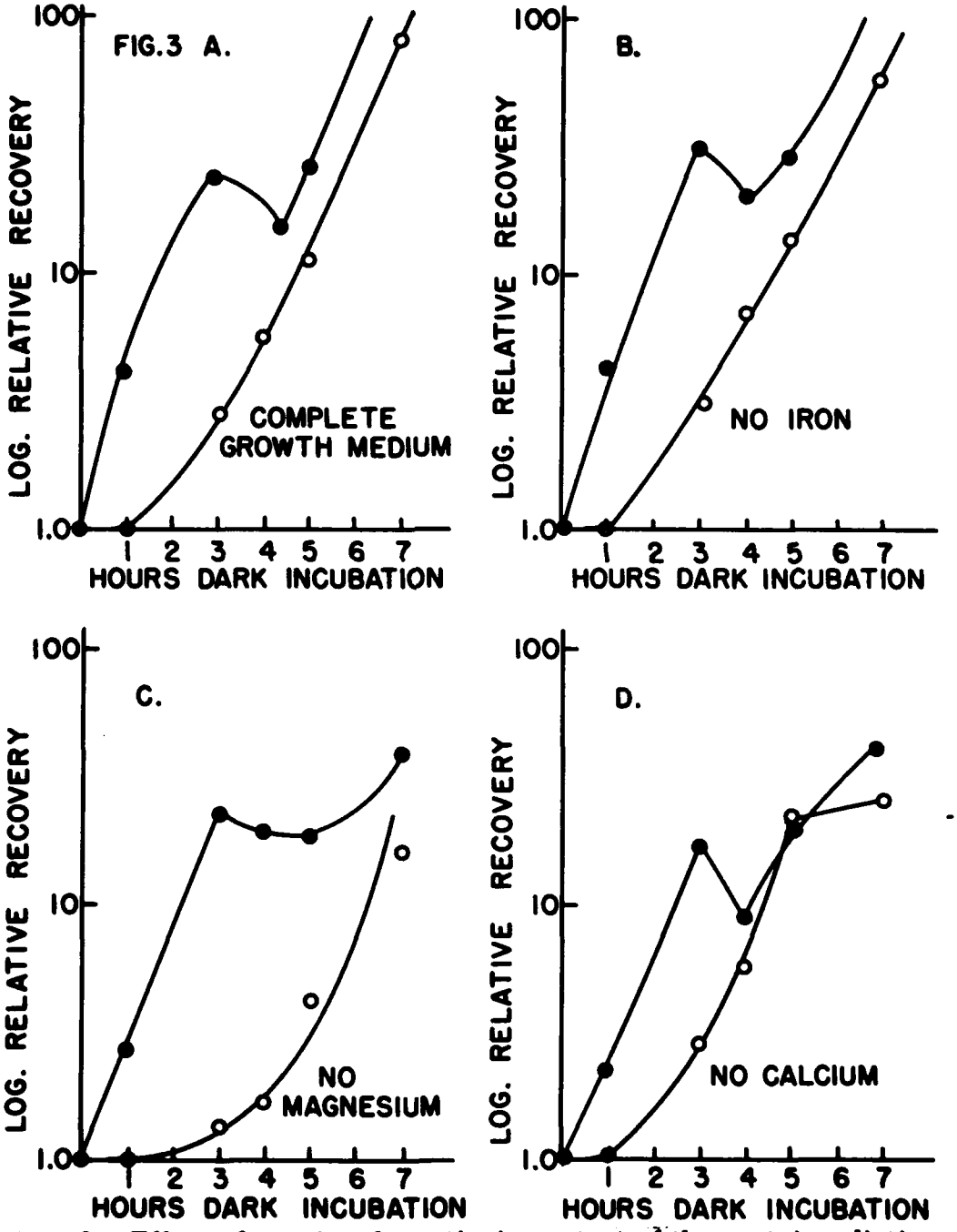


Fig. 3. Effect of varying the cationic content of the post-irradiation medium on the dark recovery of E. coli B.

O - Non-irradiated Controls **● - Irradiated Samples**

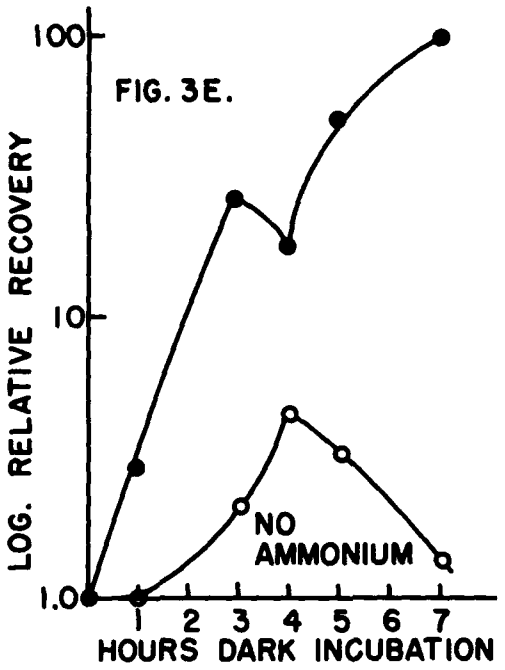
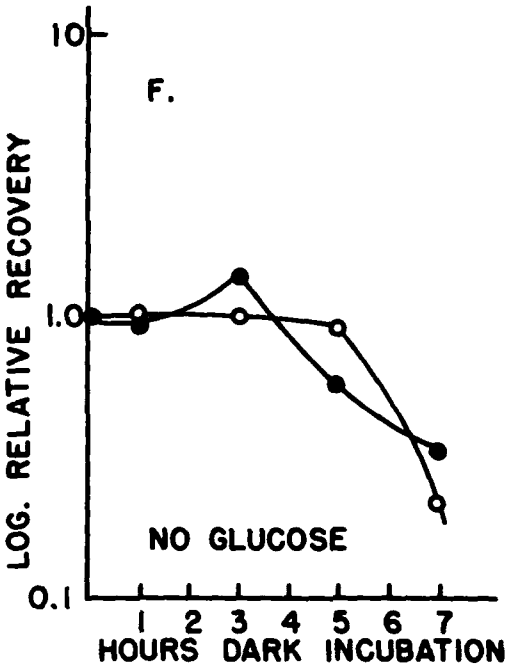
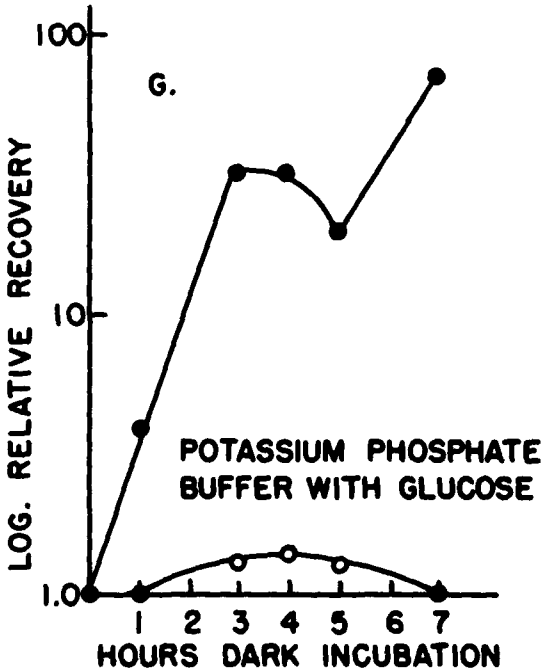
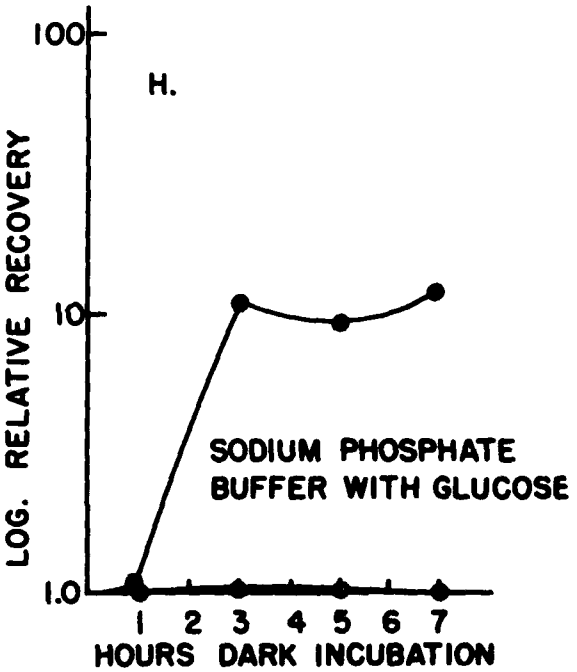


FIG. 3E.
100
10
1.0
LOG. RELATIVE RECOVERY
NO
AMMONIUM
1 2 3 4 5 6 7
HOURS DARK INCUBATION


F.
10
1.0
0.1
LOG. RELATIVE RECOVERY
NO GLUCOSE
1 2 3 4 5 6 7
HOURS DARK INCUBATION


G.
100
10
1.0
LOG. RELATIVE RECOVERY
POTASSIUM PHOSPHATE
BUFFER WITH GLUCOSE
1 2 3 4 5 6 7
HOURS DARK INCUBATION


H.
100
10
1.0
LOG. RELATIVE RECOVERY
SODIUM PHOSPHATE
BUFFER WITH GLUCOSE
1 2 3 4 5 6 7
HOURS DARK INCUBATION

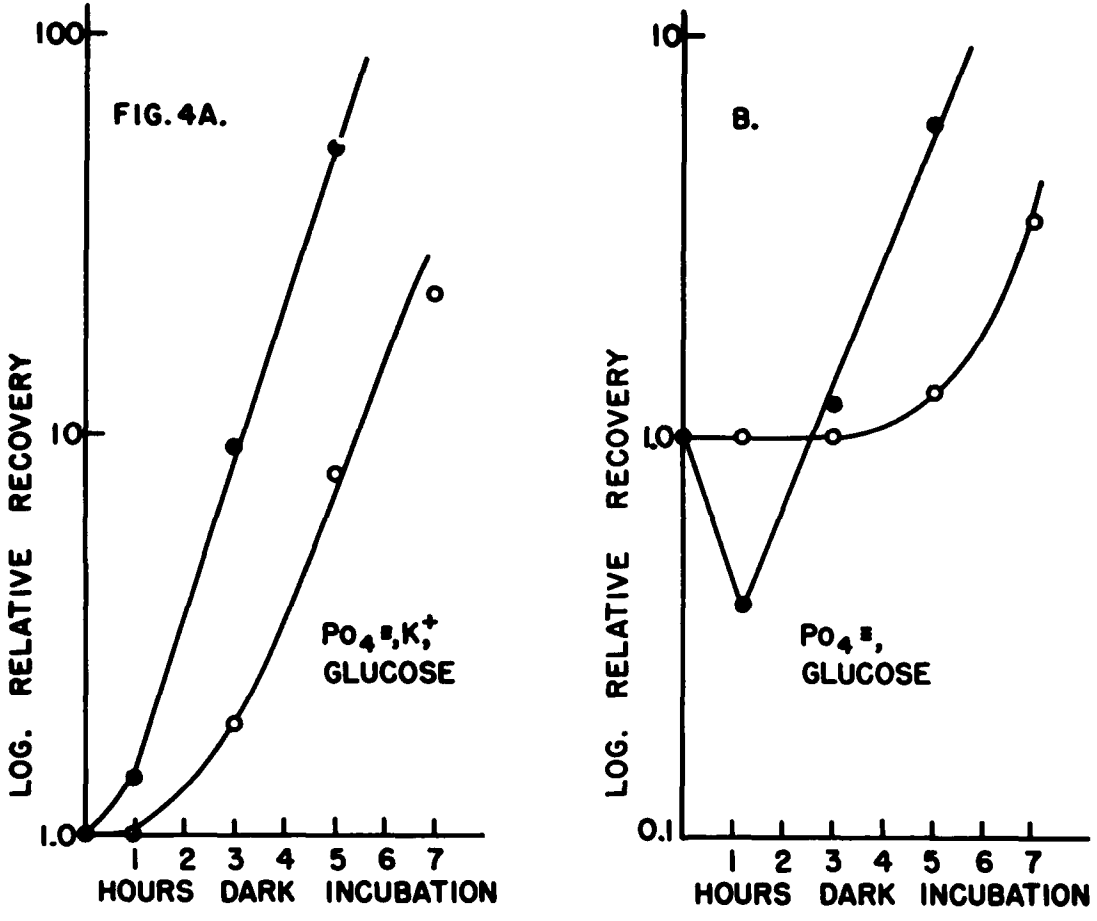


Fig. 4. Variations in the dark recovery of E. coli B when incubated in THAM buffer.

○- Non-irradiated Controls ●- Irradiated Samples